Gene expression of nutrient-sensing molecules in I cells of CCK reporter male mice

AUTHOR(S):

Kato, Tomoko; Harada, Norio; Ikeguchi-Ogura, Eri; Sankoda, Akiko; Hatoko, Tomonobu; Lu, Xuejing; Yasuda, Takuma; Yamane, Shunsuke; Inagaki, Nobuya

CITATION:

Kato, Tomoko ...[et al]. Gene expression of nutrient-sensing molecules in I cells of CCK reporter male mice. Journal of Molecular Endocrinology 2021, 66(1): 11-22

ISSUE DATE:

2021-01

URL:

http://hdl.handle.net/2433/269204

Gene expression of nutrient-sensing molecules in I cells of CCK reporter male mice

Tomoko Kato, Norio Harada, Eri Ikeguchi-Ogura, Akiko Sankoda, Tomonobu Hatoko, Xuejing Lu, Takuma Yasuda, Shunsuke Yamane, Nobuya Inagaki

Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Corresponding author

Norio Harada, M.D., Ph.D.

Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan

Tel: 81-75-751-3560, Fax: 81-75-771-4244, E-mail: nharada@kuhp.kyoto-u.ac.jp

Short title: Gene expression in I cell of CCK reporter mice

Key words: cholecystokinin, I cell, free fatty acid receptor, glucose transporter, peptide receptor

4041 words

Abbreviations:

CCK: Cholecystokinin

GIP: Glucose-dependent insulinotropic polypeptide / gastric inhibitory polypeptide

GLP-1: Glucagon-like peptide-1

FFAR: Free fatty acid receptor

GPR: G protein-coupled receptor

FATP: Fatty acid transport protein

CD36: Cluster of differentiation 36

SGLT1: Sodium-glucose cotransporter 1

GLUT: Glucose transporter

TGR5: Transmembrane GPR 5

PEPT1: Peptide transporter 1

CASR: Calcium-sensing receptor

Abstract

Cholecystokinin (CCK) is secreted from enteroendocrine I cells in response to fat, carbohydrate, and protein ingestion. Gene expression of nutrient-sensing molecules in I cells remains unclear, primarily due to the difficulty in distinguishing I cells from intestinal epithelial cells *in vivo*. In this study, we generated CCK reporter male mice in which the red fluorescence protein tdTomato (Tomato) is produced by activation of the native murine *Cck* promoter. Fluorescence microscopy revealed the presence of Tomato-positive cells in upper small intestine (SI), lower SI, and colon. Flow cytometer analysis revealed that Tomato-positive cells among epithelial cells of upper SI, lower SI, and colon occurred at the rate of 0.95%, 0.54%, and 0.06%, respectively. In upper SI and lower SI, expression levels of *Cck* mRNA were higher in Tomato-positive cells than those in Tomato-negative cells. The fatty acid receptors *Gpr120*, *Gpr40*, and *Gpr43* and the oleoylethanolamide receptor *Gpr119* were highly expressed in Tomato-positive cells isolated from SI, but were not found in Tomato-positive cells from colon. The glucose and fructose transporters *Sglt1*, *Glut2*, and *Glut5* were expressed in both Tomato-positive cells and -negative cells, but these expression levels tended to be decreased in Tomato-positive cells from upper SI to colon. The peptide transporter *Pept1* and receptor *Gpr93* were expressed in both Tomato-positive cells and -negative cells, whereas *Casr* was expressed only in Tomato-positive cells isolated from SI. Thus, this transgenic mouse reveals that I cell number and gene expression in I cells vary according to region in the gastrointestinal tract.

Introduction

Gut hormones are released from enteroendocrine cells in response to nutrients, and play an important role in food intake, nutrient absorption, energy accumulation and glucose homeostasis. For example, ghrelin secreted from X/A-like cells expressed in the stomach increases food intake and body weight (Nakazato *et al.* 2001); peptide YY (PYY) and the incretin glucagon-like peptide-1 (GLP-1) released from enteroendocrine L cells inhibit food intake and reduce body weight (Davis *et al.* 1998, Batterham *et al.* 2002). In addition, glucose-dependent insulinotropic polypeptide / gastric inhibitory polypeptide (GIP) is an incretin secreted from enteroendocrine K cells, and plays an important role in obesity and insulin resistance under high-fat diet (HFD)-fed condition (Harada *et al.* 2008, Nasteska *et al.* 2014, Joo *et al.* 2017, Shimazu-Kuwahara *et al.* 2017).

Cholecystokinin (CCK) is a gut hormone secreted from enteroendocrine I cells in small intestine and colon (Fakhry *et al.* 2017), and activates the nucleus of the solitary tract through the vagus nerve system to suppress appetite and food intake (Whited *et al.* 2006). CCK-producing cells are expressed in the central nervous system, and directly inhibit food intake (D'Agostino *et al.* 2016). The OLETF rat, which has a deletion in the *CCK1 receptor* gene, shows hyperphagia and obesity (Otsuki *et al.* 1995, Tachibana *et al.* 1996). On the other hand, CCK induces secretion of bile and pancreatic lipase, which are involved in fat digestion and absorption (Rehfeld 2004). HFD-fed *Cck*-knockout mice demonstrate that inhibition of CCK signaling alleviates body weight gain and insulin resistance under HFD-fed condition (Lo *et al.* 2011). We previously reported that CCK has an important role in oil-

induced secretion of GIP, which is involved in body weight gain and insulin resistance (Sankoda *et al.* 2017). This finding shows that CCK is involved in obesity and insulin resistance under HFD-fed condition. Thus, regulation of CCK signaling or CCK secretion is a potential therapeutic target for obesity and insulin resistance.

CCK is secreted from I cells by nutrient ingestion; fat and protein strongly stimulate CCK secretion in comparison with glucose (Green *et al.* 1989, Pilichiewicz *et al.* 2007, Hutchison *et al.* 2015). Some nutrient-sensing molecules have been identified. Glucose transporters such as sodium-glucose cotransporter 1 (SGLT1) and glucose transporter 2 (GLUT2) are associated with GLP-1 and GIP secretion after glucose loading (Mace *et al.* 2012, Gorboulev *et al.* 2012). Furthermore, free fatty acid receptors (FFARs) and fatty acid transporters (FATPs) play an important role in free fatty acid sensing in gut hormone-producing cells (Poreba *et al.* 2012, Lu *et al.* 2018). Some amino acid transporters and receptors are involved in GLP-1 secretion (Diakogiannaki *et al.* 2013). In contrast, it remains unclear whether these molecules are expressed in I cells, primarily due to the difficulty in isolating them from intestinal epithelial cells. In this study, we generated CCK reporter male mice in which the red fluorescence protein (RFP) variant tdTomato (Tomato) as well as CCK is produced by activation of the native murine *Cck* promoter, and evaluated gene expression of the molecules associated with nutrient sensing in I cells expressed in the gastrointestinal (GI) tract.

Materials and Methods

Animals

CCK-internal ribosome entry site (IRES)-Cre knock-in (CCK-Cre) mice and Ai14 mice were previously generated (JAX stock #012706, #007908) (Jackson Laboratory, Bar Harbor, Maine, US) (Madisen *et al.* 2010, Taniguchi *et al.* 2011). CCK-Cre and Ai14 heterozygous (CCK-Tomato) mice, which enabled visualization of I cells by Tomato fluorescence, were generated by crossbreeding CCK-Cre homozygous mice and Ai14 homozygous mice. Ai14 heterozygous mice were used as control. Male mice at 8-13 weeks of age were used in flow cytometer analysis and immunohistochemical analysis. We performed two cohorts to evaluate the phenotype of CCK-Tomato mice. In one cohort, 8-week-old male mice were weighed weekly for 20 weeks. Non-fasting blood samples were collected from the portal vein of mice at 10 weeks of age, and plasma CCK concentrations were measured by CCK fluorescent enzyme immunoassay (EIA) kit (FEK-069-04) (Phoenix Pharmaceuticals Inc., Burlingame, CA, US). In the other cohort, male mice at 19 weeks of age were used. Oral glucose tolerance tests (OGTTs) and oral corn oil tolerance tests (OCTTs) were performed after a 16-hour fasting period. Mice were administrated glucose of 6g/kg body weight for OGTTs and corn oil of 10mL/kg body weight for OCTTs. Blood glucose levels were measured at 0, 15 (for OGTTs), 30, 60, and 120 minutes after oral glucose or oil administration by the glucose oxidase method (Sanwa Kagaku Kenkyusho, Nagoya, Japan). 60 µl blood samples were collected from peripheral blood vessels at 15 or 30 minutes after oral glucose or oil administration, and plasma insulin (Shibayagi, Shibukawa, Japan) and CCK levels (Phoenix Pharmaceuticals Inc.) were measured by EIA kit, respectively. Energy expenditure and

120 locomotor activity were measured by ARCO 2000 (ARCO System, Chiba, Japan) every 5
121 minutes over 24 hours with free access to water and diet (Kanemaru *et al.* 2020). Animal
122 care and procedures were approved by Kyoto University Animal Care Committee
123 (MedKyo15298).
124
125 ***Immunohistochemistry***
126 Stomach, upper small intestine (upper SI), lower small intestine (lower SI), and colon were
127 collected from CCK-Tomato mice and fixed by 4% paraformaldehyde. The protocol of
128 immunohistochemistry was previously described (Ikeguchi *et al.* 2018). Anti-CCK antibody
129 (CCK8-MO-167-2, 1:1000) (Frontier Institute Co., Ltd., Hokkaido, Japan), anti-RFP antibody
130 (600-401-379, 1:1000) (Rockland Immunochemicals Inc., Limerick, PA, US), and secondary
131 antibodies (Abcam, Cambridge, UK) were used. Images were taken using a fluorescence
132 microscope FSX100 (Olympus Corporation, Tokyo, Japan).
133
134 ***Isolation of Tomato-positive and -negative cells by flow cytometry***
135 The protocol to isolate fluorescence protein-producing cells from murine intestinal
136 epithelium was described previously (Suzuki *et al.* 2013). The small intestine was divided in
137 half, and the oral and rectal portions were defined as upper SI and lower SI, respectively.
138 The collected intestinal epithelial cells were filtered through a 40 µm cell strainer (Becton,
139 Dickinson and Company, Franklin Lakes, NJ, US), and phosphate buffered salts (PBS)
140 containing 4', 6-diamidino-2-phenylindole (DAPI) (Dojindo Molecular Technologies, Inc.,

Kumamoto, Japan) was added. After excluding DAPI-positive cells as dead cells or doublets, Tomato-positive cells and -negative cells were collected using FACSAria III cell sorter (Becton, Dickinson and Company). The number of Tomato-positive cells was also calculated as Tomato-positive cells / intestinal epithelial cells (%).

Quantitative reverse-transcription polymerase chain reaction (RT-PCR)

Total RNAs of sorted Tomato-positive and -negative cells were extracted with a PicoPure RNA Isolation Kit (Applied Biosystems, California, CA, US). For cDNA synthesis of sorted 2,000 Tomato-positive and -negative cells, RNA was reverse-transcribed using SuperScript II Reverse Transcriptase and Oligo(dT)12-18 (Invitrogen, Carlsbad, CA, US). SYBR Green PCR Master Mix (Applied Biosystems) was prepared for the PCR run. The mRNA expression levels were measured by quantitative real-time PCR using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems). *Ppia* was used as the internal control. Each data point was analyzed by the comparative threshold cycle method (ΔΔCt method). Primer pairs designed for evaluation of gene expression are as follows: *Glut2*, 5'-AATGGTCGCCTCATTCTTTG-3' and 5'-ATCAAGAGGGCTCCAGTCAA-3'; *Glut5*, 5'-TCATCTCTGTGTGGAAGTTG-3' and 5'-AGATCTGATCGGCGTAGTAG-3'; *Sglt1*, 5'-GTGCTGGGCTGGATATTTGT-3' and 5'-AGGCCCAAGGCTAGATTGAT-3'; *Pept1*, 5'-ATCATTGTGCTCATCGTGGC-3' and 5'-GTGCTTCAATCTCTGCTGGG-3'; *Gpr93*, 5'-GGTGCTGATGATAATGGTGCT-3' and 5'-GTAGCCAAAGGCCTGGTATTC-3'; *Casr*, 5'-GCATCAGGTATAACTTCCGTGG-3' and 5'-TTGGAGACGGTGTTACAGGTG-3'; *Gpr41*, 5'-

TTCTTGCAGCCACACTGCTC-3' and 5'-GCCCACCACATGGGACATAT-3'; *Gpr43*, 5'-ACAGTGGAGGGGACCAAGAT-3' and 5'-GGGGACTCTCTACTCGGTGA-3'; *Gpr40*, 5'-TTTGCGCTGGGCTTTCC-3' and 5'-GCTGGGAGTGAGTCGCAGTT-3'; *Gpr119*, 5'-AGAAAGCGCCTATCACATCG-3' and 5'-CAACCTGCCTTTACCAGTTG-3'; *Cd36*, 5'-CGCTTTCTGCGTATCGTCTG-3' and 5'-GATGCACGGGATCGTGTCT-3'; *Fatp1*, 5'-TCTGTTCTGATTCGTGTTCGG-3' and 5'-AAGATGCACGGGATCGTGTC-3'; *Fatp2*, 5'-TCCTCCAAGATGTGCGGTACT-3' and 5'-TAGGTGAGCGTCTCGTCTCG-3'; *Fatp3*, 5'-ATGACAGGGGAGCCTATTCG-3' and 5'-ATCCTTCAGCAGCTTGTCCT-3'; *Fatp4*, 5'-ACTGTTCTCCAAGCTAGTGCT-3' and 5'-GATGAAGACCCGGATGAAACG-3'; *Fatp5*, 5'-CTACGCTGGCTGCATATAGATG-3' and 5'-CCACAAAGGTCTCTGGAGGAT-3', *Secretin*, 5'-AGCCCTTAGAGGACCAGCTC-3' and 5'-TGAACGATCAACAGCAGACC-3', *Glp-1*, 5'-TGAAGACAAACGCCACTCAC-3' and 5'-TCATGACGTTTGGCAATGTT-3'. Others were previously designed (Iwasaki *et al.* 2015, Sankoda *et al.* 2017).

Statistical analysis

Results are shown as dot plot or mean ± SEM. One or two data points of some results that exceeded mean ± 2SD were excluded. Statistical significance was determined by Student's t-test or one way analysis of variance with Tukey or Games-Howell test. *P* values < 0.05 were considered statistically significant.

Results

183 ***Phenotype of CCK-Tomato mice***

184 IRES and Cre recombinase were inserted downstream of the murine *CCK* locus in CCK-
185 Cre mice (Taniguchi *et al.* 2011). With this construction, the promoter and coding region of
186 both *Cck* genes were intact in CCK-Tomato mice. Body weight of the CCK-Tomato mice was
187 similar to that of control mice during 9-29 weeks of age (Fig. 1A). There was no significant
188 difference in non-fasting CCK levels between control and CCK-Tomato mice (Control mice
189 194.9±102.1mg/dl vs. CCK-Tomato mice 328.1±248.1mg/dl; P=0.10). There was no
190 significant difference in food intake, energy expenditure and locomotor activity between
191 CCK-Tomato mice and control mice (Fig. 1B and 1C). During OGTTs and OCTTs, blood glucose
192 levels were not different between the two types of mice (Fig. 1D and 1E). Plasma insulin and
193 CCK levels after glucose or corn oil administration were not different between the two
194 groups. These results indicated that the *CCK-IRES-Cre* allele does not affect body weight gain,
195 food intake, energy expenditure, locomotor activity, and glucose tolerance under 11% fat-
196 containing diet-fed condition.

197

198 ***Number of I cells in CCK-Tomato mice***

199 Under fluorescence microscopy, Tomato-positive cells were detected in upper SI,
200 lower SI, and colon of CCK-Tomato mice, but not in stomach (data not shown).
201 Immunohistochemical analysis showed that Tomato-expressing cells were identical to CCK-
202 expressing cells in upper SI, lower SI, and colon of CCK-Tomato mice (Fig. 2A).

We then evaluated the number of Tomato-expressing cells in small intestine and colon by histological analysis. The length of villus and the number of Tomato-expressing cells in small intestine were greater than those in colon (Fig. 2B and 2C). The ratio of Tomato-expressing cell number / length of villus was significantly higher in upper SI and lower SI than that in colon (Fig. 2D). In addition, the number of Tomato-positive cells was calculated by flow cytometry system (Fig. 2E). Tomato-positive cells / epithelial cells in upper SI, lower SI, and colon was 0.95 ± 0.30%, 0.54 ± 0.14%, and 0.06 ± 0.01%, respectively. Tomato-positive cell number was greater in upper SI and lower SI than that in colon, but the number significantly differed only between lower SI and colon. After purification of each 2,000 Tomato-positive and -negative cells, gene expression of *Cck* mRNA in the cells was evaluated (Fig. 2F). In upper SI, lower SI and colon, *Cck* mRNA expression was detected in Tomato-positive cells but not in Tomato-negative cells. In upper SI and lower SI, expression levels of *Cck* mRNA were higher in Tomato-positive cells than those in Tomato-negative cells. On the other hand, there was no significant difference in *Cck* mRNA expression levels between Tomato-positive and -negative cells in colon. *Cck* mRNA expression levels in Tomato-positive cells of upper SI and lower SI were significantly higher than those of colon.

Gene expression of molecules involved in fatty acid sensing in I cells

We then evaluated gene expression of G protein-coupled receptors (GPRs) and transporters for free fatty acid. In upper SI and lower SI, expression levels of the long-chain fatty acid (LCFA) receptors *Ffar4* (*Gpr120*) (Fig. 3A) and *Ffar1* (*Gpr40*) (Fig. 3B) and the

oleoylethanolamide (OEA) receptor *Gpr119* (Fig. 3C) mRNA were significantly higher in Tomato-positive cells than those in Tomato-negative cells. The expression levels did not differ between Tomato-positive and -negative cells in colon. In upper and lower SI, expression levels of the short-chain fatty acid (SCFA) receptor *Ffar2* (*Gpr43*) mRNA (Fig. 3D) were high in Tomato-positive cells compared to those in Tomato-negative cells, but expression levels of *Ffar3* (*Gpr41*) mRNA (Fig. 3E) did not differ between Tomato-positive and -negative cells. *Bile acid receptor transmembrane GPR 5* (*Tgr5*) mRNA was highly expressed in Tomato-positive cells compared to that in Tomato-negative cells in lower SI (Fig. 3F). Gene expression of the fatty acid transport protein (FATP) 1-5 and cluster of differentiation 36 (CD36) was also evaluated. *Fatp4* and *Cd36* mRNA expressions were detected in Tomato-positive cells, but the expression levels did not differ between Tomato-positive cells and -negative cells (data not shown). *Fatp1*, *Fatp2*, *Fatp3*, and *Fatp5* expressions were not detected in Tomato-positive or -negative cells (data not shown).

Gene expression of molecules involved in glucose, fructose, and amino acid sensing in I cells

In upper SI, expression levels of glucose transporters *Sglt1* (Fig. 4A) and *Glut2* (Fig. 4B) and fructose transporter *Glut5* (Fig. 4C) mRNA tended to be higher in Tomato-positive cells than those in Tomato-negative cells, but there was not a significant difference between the two groups. Expression levels of *Sglt1*, *Glut2*, and *Glut5* mRNA tended to be higher in

upper SI than those in lower SI. In colon, these expressions were not detected in Tomato-positive cells.

Gene expression levels of *peptide transporter 1* (*Pept1*) (Fig. 4D) and *Gpr93* (Fig. 4E), which are protein metabolite-sensing molecules, did not differ between Tomato-positive and -negative cells in upper and lower SI. In colon, *Gpr93* mRNA was not detected in Tomato-positive or -negative cells, whereas *Pept1* mRNA was highly expressed in Tomato-negative cells compared to that in Tomato-positive cells. mRNA of the *calcium-sensing receptor* (*Casr*), which is reported to be involved in amino acid-induced gut hormone secretion (Mace *et al.* 2012), was highly expressed in Tomato-positive cells of upper SI (Fig. 4F). On the other hand, *Casr* mRNA was not detected in Tomato-negative cells.

Gene expression of gut hormones in I cells

Some gut hormones are reported to be co-expressed in enteroendocrine cells (Egerod *et al* 2012, Habib *et al* 2012). In addition, nutrient-sensing molecules are reported to be expressed in glucose-dependent insulinotropic polypeptide / gastric inhibitory polypeptide (GIP)-producing K cells and glucagon-like peptide-1 (GLP-1)-producing L cells and to be involved in nutrient-induced GIP and GLP-1 secretion (Iwasaki *et al* 2015, Reimann *et al* 2012). We therefore evaluated mRNA expression of other gut hormones in Tomato-positive cells and -negative cells. *Secretin* and *Gip* were found to be highly expressed in Tomato-positive cells of upper SI and lower SI (Fig. 5A and 5B). On the other hand, *Glp-1* was expressed in I cells of upper SI and colon (Fig. 5C). These three gut hormones were not

265 expressed in Tomato-negative cells of SI and colon. These results indicate that some gut
266 hormone-producing cells overlap with I cells.
267
268 **Discussion**
269 In previous studies, characterization of I cells was done using purified cells from the
270 intestine of transgenic (CCK-GFP Tg) mice expressing green fluorescence protein (GFP)
271 under control of a *Cck* promoter derived from a BAC clone (Liou *et al.* 2011). Although
272 expression of some molecules associated with nutrient sensing in I cells has been
273 reported, these analyses focused on I cells expressed in small intestine. We have
274 established CCK-Tomato mice in which Tomato is expressed under endogenous and native
275 *Cck* promoter; the present study is the first to report I cell number and the expression of
276 CCK and various molecules associated with nutrient sensing in I cells of each part of the GI
277 tract.
278 I cells in the GI tract have previously been evaluated by immunohistochemistry with
279 anti-CCK antibodies. I cells are distributed throughout the small intestine and large intestine,
280 but the number of I cells in the small intestine is greater (Fakhry *et al.* 2017). Our findings
281 regarding I cell number in the GI tract of CCK-Tomato mice by immunohistochemistry with
282 anti-RFP antibodies are consistent with previous studies. However, CCK-Tomato mice
283 enabled evaluation of not only I cell number in the GI tract, but also *Cck* gene expression in
284 isolated I cells. Using the flow cytometry system, I cells among epithelial cells of upper SI,
285 lower SI, and colon were found to occur at the rate of 0.95 ± 0.30%, 0.54 ± 0.14%, and 0.06

286 ± 0.01%, respectively. A majority of the I cells were detected in upper SI, and their frequency
287 decreased toward the distal part of the GI tract. The expression levels of *Cck* mRNA in
288 isolated I cells from upper SI were highest in the GI tract, and decreased toward the distal
289 part of the GI tract. These results indicate that I cells in upper SI are the main contributor to
290 CCK secretion in response to various nutrients.

291 Fat ingestion strongly stimulates CCK secretion (Green *et al.* 1989). GPR40, GPR120,
292 and GPR119 are receptors activated by the nutrients LCFAs and OEA. These receptors are
293 reported to be expressed in incretin-producing cells and to be involved in incretin secretion
294 (Iwasaki *et al.* 2015, Sankoda *et al.* 2019). Bile, which is composed of bile acids, is important
295 for fat digestion and absorption, and is reported to induce CCK secretion in the fasting state
296 (Meyer-Gerspach *et al.* 2013). TGR5, a bile acid receptor, is expressed in L cells and is
297 involved in GLP-1 secretion (Brighton *et al.* 2015). Previous studies using the mouse
298 intestinal cell line STC-1 and *GPR40*- or *GPR120*-knockout mice showed that GPR40 and
299 GPR120 are involved in CCK secretion in response to LCFAs and fat (Tanaka *et al.* 2008,
300 Sankoda *et al.* 2017). On the other hand, it remains unclear whether GPR119 and TGR5 are
301 involved in CCK secretion, although these receptors are expressed in I cells of the small
302 intestine of CCK-GFP Tg mice (Sykaras *et al.* 2012). In our study, *Gpr40*, *Gpr120*, and *Gpr119*
303 were found to be expressed mainly in Tomato-positive cells of upper and lower SI and were
304 not detected in the cells of colon. *Tgr5* was highly expressed in Tomato-positive cells of
305 lower SI. These results indicate that these receptors may well be involved in CCK secretion
306 upon fat ingestion. Indeed, some LCFA transporters are expressed in various tissues

307 including intestine. We evaluated gene expression of *Fatp1-5* and *Cd36* mRNA in Tomato-
308 positive cells and -negative cells, and found that *Fatp4* and *Cd36* are expressed in Tomato-
309 positive cells as well as -negative cells. Our data is consistent with the previous report
310 showing that FATP4 and CD36 are involved in GLP-1 and CCK secretion, respectively (Poreba
311 *et al.* 2012, Sundaresan *et al.* 2013), and go further to suggest their role in CCK secretion
312 upon fat ingestion.

313 Glucose and fructose are known to induce CCK secretion (Kuhre *et al.* 2014); the
314 glucose transporters SGLT1 and GLUT2 and fructose transporter GLUT5 are expressed in
315 enteroendocrine cells and are involved in glucose and fructose-induced gut hormone
316 secretion, respectively (Reimann *et al.* 2008, Parker *et al.* 2009, Mace *et al.* 2012, Gorboulev
317 *et al.* 2012). The previous study using small intestine of CCK-GFP Tg mice revealed that
318 SGLT1 is expressed in I cells as well as other intestinal epithelial cells (Kaelberer *et al*. 2018),
319 but the expression of GLUT2 and GLUT5 in I cells was not examined. In the present study,
320 *Sglt1*, *Glut2*, and *Glut5* were found to be expressed in both Tomato-positive cells and -
321 negative cells in upper SI, lower SI, and colon, and the expression levels tended to be higher
322 in upper SI than those in lower SI and colon. These results revealed that the expression
323 patterns in Tomato-positive and -negative cells in SI and colon are similar among the three
324 transporters.

325 Peptide transporter PEPT1, peptone receptor GPR93, and amino acid-sensing
326 receptor CASR have been identified as protein metabolite-sensing molecules associated
327 with gut hormone secretion (Feng *et al.* 2010, Mace *et al.* 2012, Diakogiannaki *et al.* 2013).

In vitro and *in vivo* studies have shown that GPR93 and CASR are involved in amino acid-induced CCK secretion from I cells (Choi *et al.* 2007, Liou *et al.* 2011); PEPT1, GPR93, and CASR have been reported to be expressed in I cells of small intestine of CCK-GFP Tg mice (Liou *et al.* 2011). In the present study, these molecules were found to be expressed in Tomato-positive cells of upper and lower SI; *Pept1* and *Gpr93* were found to be expressed in both Tomato-positive and -negative cells. On the other hand, *Casr* was expressed in Tomato-positive cells but not in Tomato-negative cells, indicating that *Casr* is specifically expressed in I cells. In colon, expression levels of *Pept1* mRNA were significantly higher in Tomato-negative cells than those in Tomato-positive cells. As PEPT1 is reported to play an important role in the regulation of water absorption into intestinal epithelium (Wuensch *et al.* 2013), the molecule might be highly expressed in intestinal epithelial cells detected as Tomato-negative cells.

CCK plays a key physiological role in fat absorption and regulation of energy intake. Thus, regulation of nutrient-sensing molecule-mediated CCK secretion might represent possible novel therapeutic approaches to obesity and type 2 diabetes. Analysis using CCK-Tomato mice revealed that I cells are broadly distributed throughout the GI tract, and that the various molecules involved in nutrient sensing are abundantly expressed in I cells.

Declaration of interests

N. I. received joint research grants from Daiichi Sankyo Co., Ltd., Terumo Co., Ltd., and Drawbridge, Inc.; received speaker honoraria from Kowa Pharmaceutical Co., Ltd.; MSD,

Astellas Pharma Inc., Novo Nordisk Pharma Ltd., Ono Pharmaceutical Co., Ltd., Nippon Boehringer Ingelheim Co., Ltd., Takeda Pharmaceutical Co., Ltd., and Mitsubishi Tanabe Pharma Co., Ltd.; received scholarship grant from Kissei Pharmaceutical Co., Ltd., Sanofi , Daiichi Sankyo Co., Ltd., Mitsubishi Tanabe Pharma Co., Ltd., Takeda Pharmaceutical Co., Ltd., Japan Tobacco Inc., Kyowa Kirin Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Astellas Pharma Inc., MSD, Eli Lilly Japan, Ono Pharmaceutical Co. Ltd., Sanwa Kagaku Kenkyusho Co. Ltd., Nippon Boehringer Ingelheim Co., Ltd., Novo Nordisk Pharma Ltd., Novartis Pharma K.K., Teijin Pharma Ltd., and Life Scan Japan Inc.. N. H. received scholarship grant from Mitsubishi Tanabe Pharma Co., Ltd., Ono Pharmaceutical Co. Ltd., Sanofi, and Novo Nordisk Pharma Ltd. All other authors have nothing to disclose.

Author contribution statement

T.K. and N.H. planned the study, researched data, contributed to discussion, wrote, reviewed and edited the manuscript. E.I-O., A.S., T.H., X.L., T.Y., and S.Y. researched data. N.I. planned the study, contributed to discussion, and edited the manuscript. All authors approved the final version of the manuscript.

Funding

This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan Society for the Promotion of Science (JSPS) (grant numbers 20H03731, 19K09022), Ministry of Health, Labour, and Welfare, Ministry of Agriculture,

Forestry and Fisheries, Japan Diabetes Foundation, Japan Association for Diabetes Education and Care, Merck Sharp & Dohme (MSD) Life Science Foundation, Public Interest Incorporated Foundation, and Japan Diabetes Foundation.

Acknowledgements

The authors thank the Medical Research Support Center, Graduate School of Medicine, Kyoto University for experimental support.

References

Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ & Ghatei MA et al. 2002 Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* **418** 650-654. (doi:10.1038/nature00887)

Brighton CA, Rievaj J, Kuhre RE, Glass LL, Schoonjans K, Holst JJ, Gribble FM & Reimann F 2015 Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein-coupled bile acid receptors. *Endocrinology* **156** 3961-3970. (doi:10.1210/en.2015-1321)

Choi S, Lee M, Shiu AL, Yo SJ, Halldén G & Aponte GW 2007 GPR93 activation by protein hydrolysate induces CCK transcription and secretion in STC-1 cells. *The American Journal of Physiology-Gastrointestinal and Liver Physiology* **292** G1366-1375. (doi:10.1152/ajpgi.00516.2006)

D'Agostino G, Lyons DJ, Cristiano C, Burke LK, Madara JC, Campbell JN, Garcia AP, Land BB, Lowell BB & Dileone RJ et al. 2016 Appetite controlled by a cholecystokinin nucleus of the solitary tract to hypothalamus neurocircuit. *eLife* **5** e12225. (doi:10.7554/eLife.12225)

Davis HR Jr, Mullins DE, Pines JM, Hoos LM, France CF, Compton DS, Graziano MP, Sybertz EJ, Strader CD & Van Heek M 1998 Effect of chronic central administration of glucagon-like peptide-1 (7-36) amide on food consumption and body weight in normal and obese rats. *Obesity Research* **6** 147-156. (doi:10.1002/j.1550-8528.1998.tb00329.x)

Diakogiannaki E, Pais R, Tolhurst G, Parker HE, Horscroft J, Rauscher B, Zietek T, Daniel H, Gribble FM & Reimann F 2013 Oligopeptides stimulate glucagon-like peptide-1 secretion in

mice through proton-coupled uptake and the calcium-sensing receptor. *Diabetologia* **56** 2688-2696. (doi:10.1007/s00125-013-3037-3)

Dyer J, Varro A, Dockray GJ & Shirazi-Beechey SP 1997 The expression of sugar transporters in the endocrine cell line STC-1. *Biochemical Society Transactions* **25** 478S. (doi:10.1042/bst025478s)

Egerod KL, Engelstoft MS, Grunddal KV, Nøhr MK, Secher A, Sakata I, Pedersen J, Windeløv JA, Füchtbauer EM & Olsen J et al. 2012 A major lineage of enteroendocrine cells coexpress CCK, secretin, GIP, GLP-1, PYY, and neurotensin but not somatostatin. *Endocrinology* **153** 5782-5795. (doi:10.1210/en.2012-1595)

Fakhry J, Wang J, Martins P, Fothergill LJ, Hunne B, Prieur P, Shulkes A, Rehfeld JF, Callaghan B & Furness JB 2017 Distribution and characterisation of CCK containing enteroendocrine cells of the mouse small and large intestine. *Cell and Tissue Research* **369** 245-253. (doi:10.1007/s00441-017-2612-1)

Feng J, Petersen CD, Coy DH, Jiang JK, Thomas CJ, Pollak MR & Wank SA 2010 Calcium-sensing Receptor Is a Physiologic Multimodal Chemosensor Regulating Gastric G-cell Growth and Gastrin Secretion. *Proceedings of the National Academy of Sciences of the United States of America* **107** 17791-17796. (doi:10.1073/pnas.1009078107)

Gorboulev V, Schürmann A, Vallon V, Kipp H, Jaschke A, Klessen D, Friedrich A, Scherneck S, Rieg T, Cunard R et al. 2012 Na(+)-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. *Diabetes* **61** 187-196. (doi:10.2337/db11-1029)

421 Green GM, Taguchi S, Friestman J, Chey WY & Liddle RA 1989 Plasma secretin, CCK, and
422 pancreatic secretion in response to dietary fat in the rat. *The American Journal of Physiology*
423 **256** G1016-1021. (doi:10.1152/ajpgi.1989.256.6.G1016)
424 Habib AM, Richards P, Cairns LS, Rogers GJ, Bannon CAM, Parker HE, Morley TCE, Yeo GSH,
425 Reimann F & Gribble FM 2012 Overlap of endocrine hormone expression in the mouse
426 intestine revealed by transcriptional profiling and flow cytometry. *Endocrinology* **153** 3054-
427 3065. (doi:10.1210/en.2011-2170)
428 Harada N, Yamada Y, Tsukiyama K, Yamada C, Nakamura Y, Mukai E, Hamasaki A, Liu X,
429 Toyoda K & Seino Y et al. 2008 A novel GIP receptor splice variant influences GIP sensitivity
430 of pancreatic beta-cells in obese mice. *The American Journal of Physiology-Endocrinology*
431 *and Metabolism* **294** E61-68. (doi:10.1152/ajpendo.00358.2007)
432 Hutchison AT, Feinle-Bisset C, Fitzgerald PC, Standfield S, Horowitz M, Clifton PM &
433 Luscombe-Marsh ND 2015 Comparative effects of intraduodenal whey protein hydrolysate
434 on antropyloroduodenal motility, gut hormones, glycemia, appetite, and energy intake in
435 lean and obese men. *The American Journal of Clinical Nutrition* **102** 1323-1331.
436 (doi:10.3945/ajcn.115.114538)
437 Ikeguchi E, Harada N, Kanemaru Y, Sankoda A, Yamane S, Iwasaki K, Imajo M, Murata Y,
438 Suzuki K & Joo E et al. 2018 Transcriptional factor Pdx1 is involved in age-related GIP
439 hypersecretion in mice. *The American Journal of Physiology-Gastrointestinal and Liver*
440 *Physiology* **315** G272-G282. (doi:10.1152/ajpgi.00054.2018)

Iwasaki K, Harada N, Sasaki K, Yamane S, Iida K, Suzuki K, Hamasaki A, Nasteska D, Shibue K & Joo E et al. 2015 Free fatty acid receptor GPR120 is highly expressed in enteroendocrine K cells of the upper small intestine and has a critical role in GIP secretion after fat ingestion. *Endocrinology* **156** 837-846. (doi:10.1210/en.2014-1653)

Joo E, Harada N, Yamane S, Fukushima T, Taura D, Iwasaki K, Sankoda A, Shibue K, Harada T & Suzuki K et al. 2017 Inhibition of Gastric Inhibitory Polypeptide Receptor Signaling in Adipose Tissue Reduces Insulin Resistance and Hepatic Steatosis in High-Fat Diet-Fed Mice. *Diabetes* **66** 868-879. (doi:10.2337/db16-0758)

Kaelberer MM, Buchanan KL, Klein ME, Barth BB, Montoya MM, Shen X, Diego V & Bohórquez DV 2018 A gut-brain neural circuit for nutrient sensory transduction. *Science* **361** eaat5236. (doi:10.1126/science.aat5236)

Kanemaru Y, Harada N, Shimazu-Kuwahara S, Yamane S, Ikeguchi E, Murata Y, Kiyobayashi S, Hatoko T & Inagaki N 2020 Absence of GIP secretion alleviates age-related obesity and insulin resistance. *Journal of Endocrinology* **245** 13-20. (doi:10.1530/JOE-19-0477)

Kuhre RE, Gribble FM, Hartmann B, Reimann F, Windeløv JA, Rehfeld JF & Holst JJ 2014 Fructose stimulates GLP-1 but not GIP secretion in mice, rats, and humans. *The American Journal of Physiology-Gastrointestinal and Liver Physiology* **306** G622-630. (doi:10.1152/ajpgi.00372.2013)

Liou AP, Lu X, Sei Y, Zhao X, Pechhold S, Carrero RJ, Raybould HE & Wank S 2011 The G-protein-coupled receptor GPR40 directly mediates long-chain fatty acid-induced secretion of cholecystokinin. *Gastroenterology* **140** 903-12. (doi:10.1053/j.gastro.2010.10.012)

462 Liou AP, Sei Y, Zhao X, Feng J, Lu X, Thomas C, Pechhold S, Raybould HE & Wank SA 2011 The
463 extracellular calcium-sensing receptor is required for cholecystokinin secretion in response
464 to L-phenylalanine in acutely isolated intestinal I cells. *The American Journal of Physiology-*
465 *Gastrointestinal and Liver Physiology* **300** G538-546. (doi:10.1152/ajpgi.00342.2010)
466 Lo CM, Obici S, Dong HH, Haas M, Lou D, Kim DH, Liu M, D'Alessio D, Woods SC & Tso P 2011
467 Impaired insulin secretion and enhanced insulin sensitivity in cholecystokinin-deficient mice.
468 *Diabetes* **60** 2000-2007. (doi:10.2337/db10-0789)
469 Lu VB, Gribble FM & Reimann F 2018 Free Fatty Acid Receptors in Enteroendocrine Cells.
470 *Endocrinology* **159** 2826-2835. (doi:10.1210/en.2018-00261)
471 Mace OJ, Schindler M & Patel S 2012 The regulation of K- and L-cell activity by GLUT2 and
472 the calcium-sensing receptor CasR in rat small intestine. *The Journal of Physiology* **590** 2917-
473 2936. (doi:10.1113/jphysiol.2011.223800)
474 Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD,
475 Hawrylycz MJ & Jones AR et al. 2010 A robust and high-throughput Cre reporting and
476 characterization system for the whole mouse brain. *Nature Neuroscience* **13** 133-140.
477 (doi:10.1038/nn.2467)
478 Meyer-Gerspach AC, Steinert RE, Keller S, Malarski A, Schulte FH & Beglinger C 2013 Effects
479 of chenodeoxycholic acid on the secretion of gut peptides and fibroblast growth factors in
480 healthy humans. *The Journal of Clinical Endocrinology & Metabolism* **98** 3351-3358.
481 (doi:10.1210/jc.2012-4109)

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K & Matsukura S 2001 A role for ghrelin in the central regulation of feeding. *Nature* **409** 194-198. (doi:10.1038/35051587)

Nasteska D, Harada N, Suzuki K, Yamane S, Hamasaki A, Joo E, Iwasaki K, Shibue K, Harada T & Inagaki N 2014 Chronic reduction of GIP secretion alleviates obesity and insulin resistance under high-fat diet conditions. *Diabetes* **63** 2332-2343. (doi:10.2337/db13-1563)

Otsuki M, Akiyama T, Shirohara H, Nakano S, Furumi K & Tachibana I 1995 Loss of sensitivity to cholecystokinin stimulation of isolated pancreatic acini from genetically diabetic rats. *The American Journal of Physiology* **268** E531-536. (doi:10.1152/ajpendo.1995.268.3.E531)

Parker HE, Habib AM, Rogers GJ, Gribble FM & Reimann F 2009 Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. *Diabetologia* **52** 289-298. (doi:10.1007/s00125-008-1202-x)

Pilichiewicz AN, Chaikomin R, Brennan IM, Wishart JM, Rayner CK, Jones KL, Smout AJ, Horowitz M & Feinle-Bisset C 2007 Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men. *The American Journal of Physiology-Endocrinology and Metabolism* **293** E743-753. (doi:10.1152/ajpendo.00159.2007)

Poreba MA, Dong CX, Li SK, Stahl A, Miner JH & Brubaker PL 2012 Role of fatty acid transport protein 4 in oleic acid-induced glucagon-like peptide-1 secretion from murine intestinal L cells. *The American Journal of Physiology-Endocrinology and Metabolism* **303** E899-907. (doi:10.1152/ajpendo.00116.2012)

503 Rehfeld JF 2004 Clinical endocrinology and metabolism. Cholecystokinin *Best Practice &*
504 *Research Clinical Endocrinology & Metabolism* **18** 569-586.
505 (doi:10.1016/j.beem.2004.07.002)
506 Reimann F, Habib AM, Tolhurst G, Parker HE, Rogers GJ & Gribble FM 2008 Glucose Sensing
507 in L Cells: A Primary Cell Study. *Cell Metabolism* **8** 532-539.
508 (doi:10.1016/j.cmet.2008.11.002)
509 Sankoda A, Harada N, Iwasaki K, Yamane S, Murata Y, Shibue K, Thewjitcharoen Y, Suzuki K,
510 Harada T & Kanemaru Y et al. 2017 Long-chain free fatty acid receptor GPR120 mediates oil-
511 Induced GIP secretion through CCK in male mice. *Endocrinology* **158** 1172-1180.
512 (doi:10.1210/en.2017-00090)
513 Sankoda A, Harada N, Kato T, Ikeguchi E, Iwasaki K, Yamane S, Murata Y, Hirasawa A & Inagaki
514 N 2019 Free fatty acid receptors, G protein-coupled receptor 120 and G protein-coupled
515 receptor 40, are essential for oil-induced gastric inhibitory polypeptide secretion. *Journal of*
516 *Diabetes Investigation* **10** 1430-1437. (doi:10.1111/jdi.13059)
517 Shimazu-Kuwahara S, Harada N, Yamane S, Joo E, Sankoda A, Kieffer TJ & Inagaki N 2017
518 Attenuated secretion of glucose-dependent insulinotropic polypeptide (GIP) does not
519 alleviate hyperphagic obesity and insulin resistance in ob/ob mice. *Molecular Metabolism* **6**
520 288-294. (doi:10.1016/j.molmet.2017.01.006)
521 Sundaresan S, Shahid R, Riehl TE, Chandra R, Nassir F, Stenson WF, Liddle RA & Abumrad NA
522 2013 CD36-dependent signaling mediates fatty acid-induced gut release of secretin and
523 cholecystokinin. *The FASEB Journal* **27** 1191-1202. (doi:10.1096/fj.12-217703)

524 Suzuki K, Harada N, Yamane S, Nakamura Y, Sasaki K, Nasteska D, Joo E, Shibue K, Harada T
525 & Hamasaki A et al. 2013 Transcriptional regulatory factor X6 (Rfx6) increases gastric
526 inhibitory polypeptide (GIP) expression in enteroendocrine K-cells and is involved in GIP
527 hypersecretion in high fat diet-induced obesity. *The Journal of Biological Chemistry* **288**
528 1929-1938. (doi:10.1074/jbc.M112.423137)
529 Sykaras AG, Demenis C, Case RM, McLaughlin JT & Smith CP 2012 Duodenal
530 enteroendocrine I-cells contain mRNA transcripts encoding key endocannabinoid and fatty
531 acid receptors. *PLOS ONE* 7 e42373. (doi:10.1371/journal.pone.0042373)
532 Tachibana I, Akiyama T, Kanagawa K, Shiohara H, Furumi K, Watanabe N & Otsuki M 1996
533 Defect in pancreatic exocrine and endocrine response to CCK in genetically diabetic OLETF
534 rats. *The American Journal of Physiology* **270** G730-737.
535 (doi:10.1152/ajpgi.1996.270.4.G730)
536 Tanaka T, Katsuma S, Adachi T, Koshimizu TA, Hirasawa A & Tsujimoto G 2008 Free fatty acids
537 induce cholecystokinin secretion through GPR120. *Naunyn-Schmiedeberg's Archives of*
538 *Pharmacology* **377** 523-527. (doi:10.1007/s00210-007-0200-8)
539 Taniguchi H, He M, Wu P, Kim S, Paik R, Sugino K, Kvitsiani D, Fu Y, Lu J & Lin Y et al. 2011 A
540 resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex.
541 *Neuron* **71** 995-1013. (doi:10.1016/j.neuron.2011.07.026)
542 Whited KL, Thao D, Kent Lloyd KC, Kopin AS & Raybould HE 2006 Targeted disruption of the
543 murine CCK1 receptor gene reduces intestinal lipid-induced feedback inhibition of gastric

544 function. *The American Journal of Physiology-Gastrointestinal and Liver Physiology* **291**
545 G156-162. (doi:10.1152/ajpgi.00569.2005)
546 Wuensch T, Schulz S, Ullrich S, Lill N, Stelzl T, Rubio-Aliaga I, Loh G, Chamaillard M, Haller D
547 & Daniel H 2013 The peptide transporter PEPT1 is expressed in distal colon in rodents and
548 humans and contributes to water absorption. *The American Journal of Physiology-*
549 *Gastrointestinal and Liver Physiology* **305** G66-73. (doi:10.1152/ajpgi.00491.2012)
550

551 **Figure legends**

552 **Figure 1. Phenotype of CCK-Tomato mice**

553 (A) Body weight, (B) food consumption, (C) energy expenditure, and locomotor activity
554 (n=5-6). Blood glucose levels and plasma insulin and CCK levels during (D) OGTTs and (E)
555 OCTTs (n=6). Control mice (white circles) and CCK-Tomato mice (black circles). $^{\#}P<0.05$,
556 $^{\#\#}P<0.01$ vs plasma glucose levels at 0 min, n.s.; not significant.

557

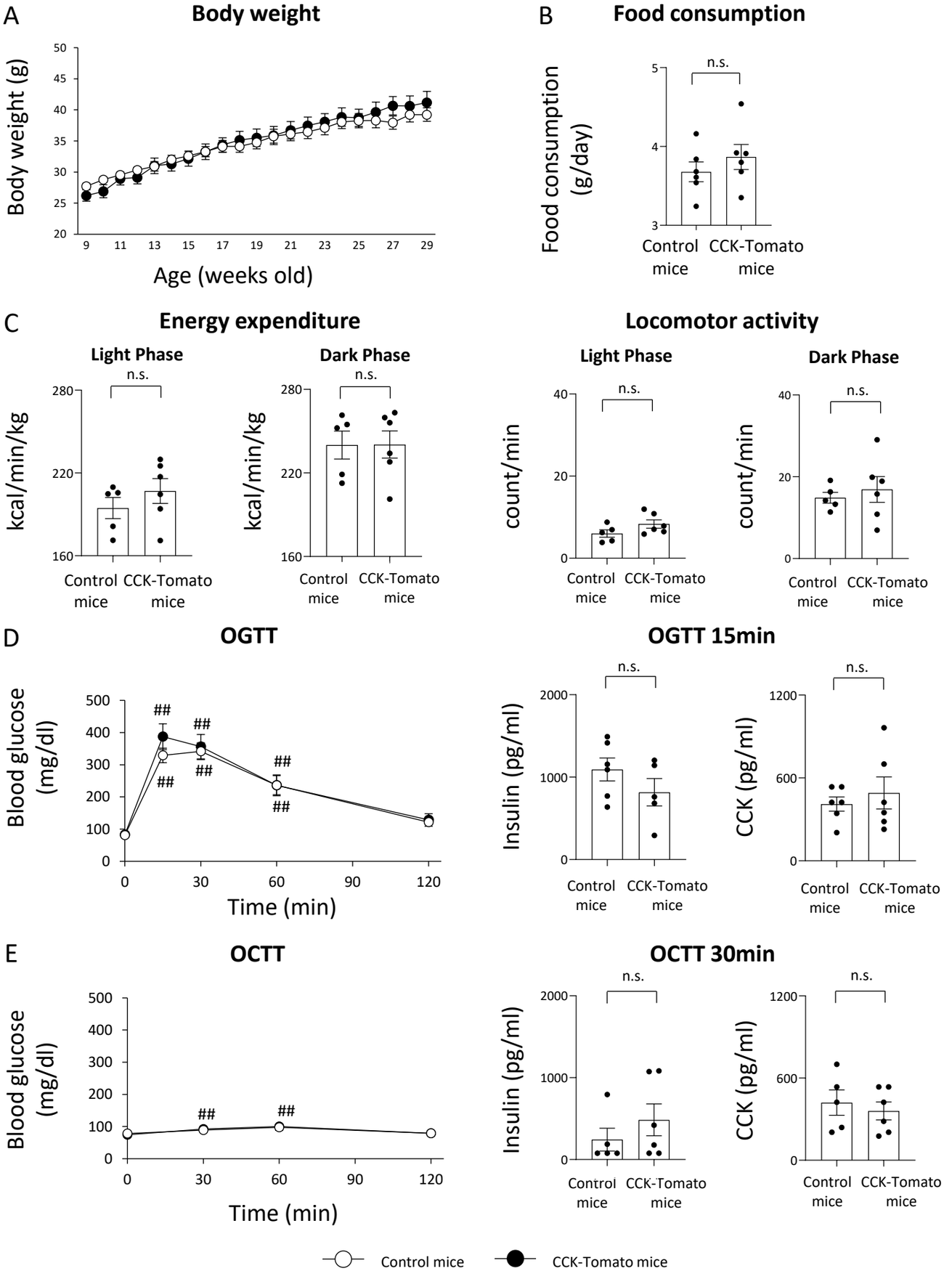
558 **Figure 2. Localization of I cells in the GI tract of CCK-Tomato mice.**

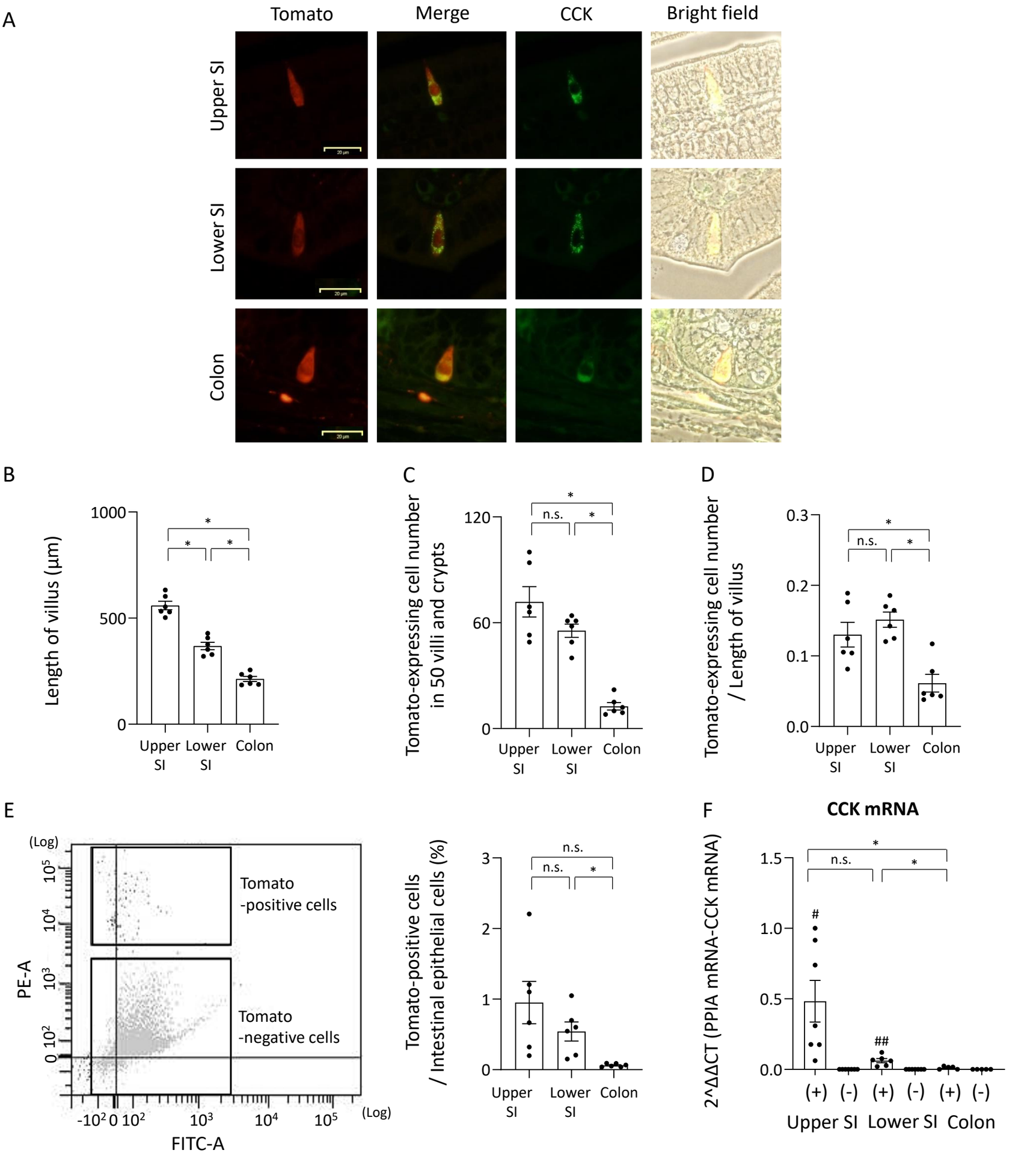
559 (A) Immunohistochemical images of the upper SI, lower SI, and colon in CCK-Tomato mice.
560 Red: Tomato-expressing cells, Green: CCK-expressing cells, Yellow: merged image. (B)
561 Length of villus and (C) number of Tomato-expressing cells in 50 villi and crypts were
562 measured by immunohistochemistry (n=6). (D) Tomato-expressing cells were quantified as
563 the number of Tomato-expressing cells / length of mucous membrane (n=6). (E) The levels
564 of red fluorescence in isolated epithelial cells were evaluated and Tomato-positive cells
565 were counted from the data of flow cytometry analysis (n=6). (F) Expression of *Cck* mRNA
566 in I cells (n=6-7). $^{\#}P<0.05$ and $^{\#\#}P<0.01$ vs. Tomato negative cells, $^{*}P<0.05$, n.s.; not
567 significant.

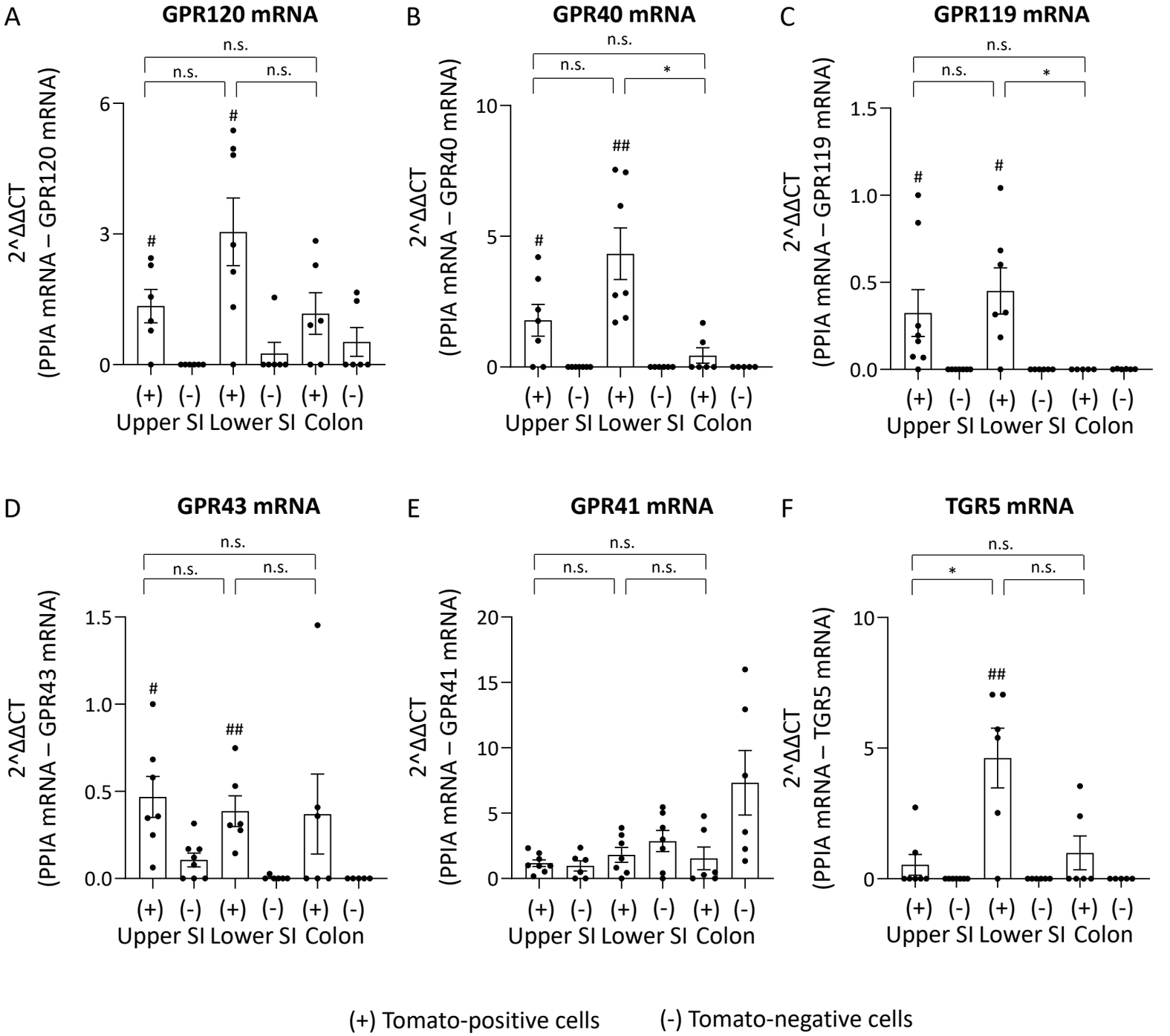
568

569 **Figure 3. Expressions of FFARs and TGR5 mRNA and in I cells.**

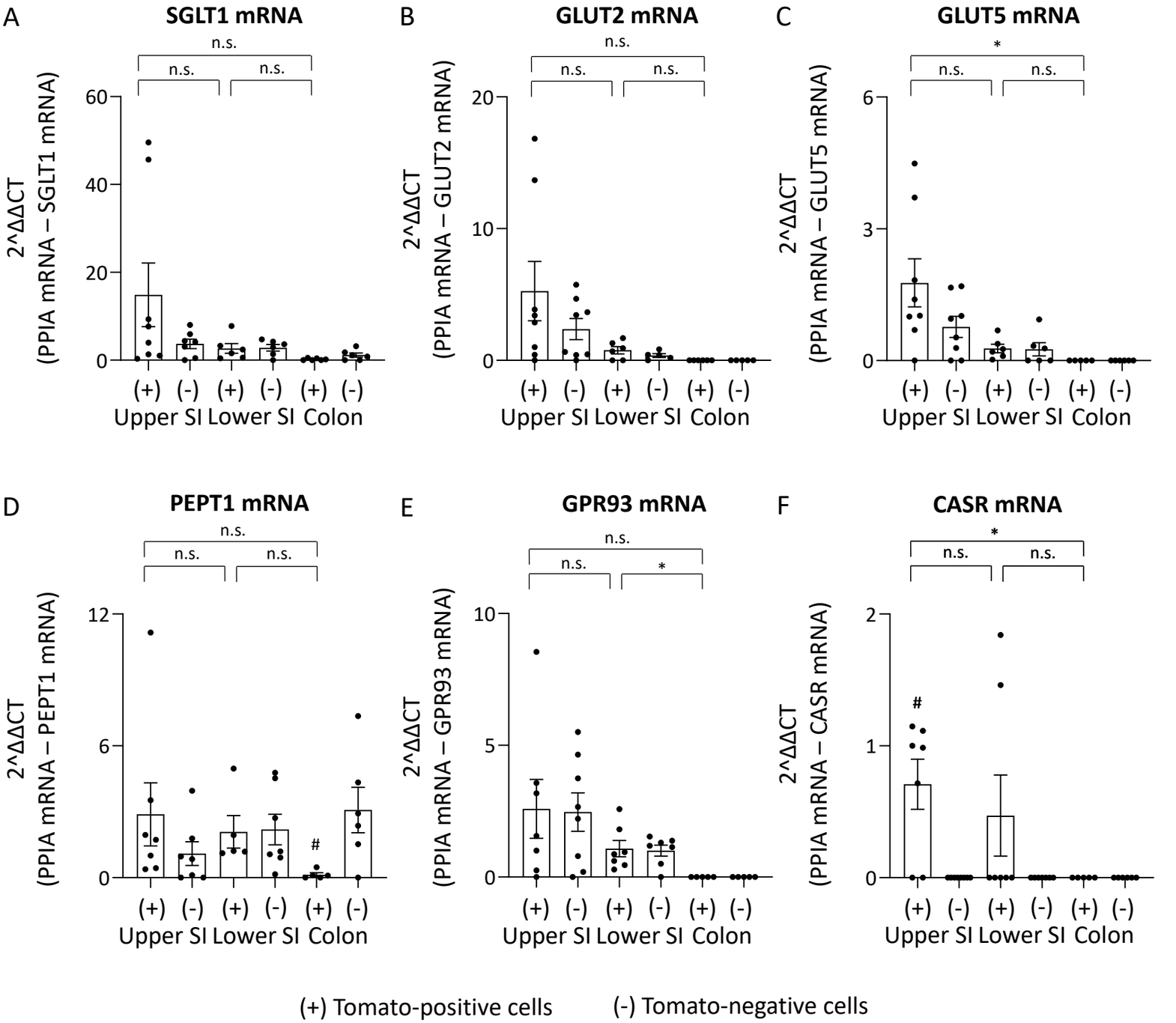
570 Data are shown as relative expression to that of *Ppia* expression in parallel in the same
571 samples (A: *Gpr120*, B: *Gpr40*, C: *Gpr119*, D: *Gpr43*, E: *Gpr41*, F: *Tgr5*) (n=6-8). $^{\#}$P<0.05 and
572 $^{\#\#}$P<0.01 vs. Tomato-negative cells, *P<0.05 and **P<0.01, n.s.; not significant.
573
574 **Figure 4. Expressions of glucose transporters, amino acid transporter, and amino acid**
575 **receptors mRNA in I cells.**
576 Data are shown as relative expression to that of PPIA expression in parallel in the same
577 samples (A: *Sglt1*, B: *Glut2*, C: *Glut5*, D: *Pept1*, E: *Gpr93*, F: *Casr*) (n=6-8). $^{\#}$P<0.05 and
578 $^{\#\#}$P<0.01 vs. Tomato-negative cells, *P<0.05 and **P<0.01, n.s.; not significant.
579
580 **Figure 5. Expressions of gut hormones mRNA in I cells.**
581 Expression levels of (A) *Secretin* mRNA, (B) *Gip* mRNA, and (C) *Glp-1* mRNA in Tomato-
582 positive and -negative cells. Data are shown as relative expression to that of PPIA expression
583 in parallel in the same samples (n=6-8). $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. Tomato-negative cells,
584 *P<0.05 and **P<0.01, n.s.; not significant.







(+) Tomato-positive cells (-) Tomato-negative cells



(+) Tomato-positive cells (-) Tomato-negative cells